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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OCT 25 1984

Releasable

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Completion of General Linuron Mutagenicity Testing Requirements. #352-326

TO: Robert Taylor, PM#25
Fungicide-Herbicide Branch
Registration Division (TS-767C)

FROM: Charles N. Aldous, Ph.D.
Toxicologist, Section V
Toxicology Branch/HED (TS-769C)

Charles N Aldous
Oct 24, 1984

THRU: Laurence D. Chitlik, DABT
Section Head, Section V & VI
Toxicology Branch/HED (TS-769C)

LDC 10/24/84

and

William L. Burnam, Chief
Toxicology Branch/HED (TS-767C)

WLB 10/25/84

Final copies of 4 mutagenicity study reviews have been received from contract reviewers as of October 9, 1984. These studies arrived under action #352-326 in two folders. One folder contained Accession #251571 and the other folder #252172. One-liners for these studies are attached . The registrants' data will be submitted separately upon receipt from the contractor.

All 4 studies are acceptable, and fill the three subcategories for mutagenicity testing of EPA data requirements of 40 CFR, Part 158.135. The specific subcategories for mutagenicity testing have been filled as follows:

Mutagenicity Testing Category	Applicable Study(ies) by MRID
Gene Mutation	00131738, 00137152
Chromosomal Aberration	00137153
Other Mechanisms of Mutagenicity	00132583

Fulfillment of these data gaps should be noted with reference to the Linuron Guidance package dated June 29, 1984 (p. 52).

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Although the generic mutagenicity requirements have been fulfilled, it should be noted that DuPont has one related study underway relevant to oncogenicity: Dr. Richard F. Holt has informed us on September 19, 1984 that a study evaluating interaction of linuron with pituitary and testicular hormone systems is in process. Note that this ancillary study relates to the most definitive oncogenic response observed in chronic studies: rat testicular adenomas.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA: 68-01-6561
TASK:62
September 6, 1984

DATA EVALUATION RECORD

LINURON

Mutagenicity

CITATION: Russell, James F. Mutagenicity evaluation in Salmonella typhimurium. Unpublished report no. 106-83, prepared by E. I. du Pont de Nemours and Co., Inc., Newark, Delaware. Dated May 5, 1983 (Revised June 8, 1983).

REVIEWED BY:

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Signature: William L. McLellan (for)
Date: 9-6-84

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APPROVED BY:

Charles Aldous, Ph.D.
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Signature: Charles N Aldous
Date: 10 Oct 1984

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity - Reverse mutation assay in S. typhimurium.

CITATION: Russell, James F. Mutagenicity evaluation in Salmonella typhimurium. Unpublished report no. 106-83, prepared by E. I. du Pont de Nemours and Co., Inc., Newark, Delaware. Dated May 5, 1983 (Revised June 8, 1983).

ACCESSION NUMBER: 251571.

MRID NUMBER: 00131738.

LABORATORY: E. I. du Pont de Nemours and Co., Inc. Haskell Laboratory for Toxicology and Industrial Medicine, Elkton Road, P. O. Box 50, Newark, Delaware 19711.

QUALITY ASSURANCE STATEMENT: Not present for this report.

TEST MATERIAL: The test material, Linuron (N'-(3,4-dichlorophenyl)-N-methoxy-N-methyl urea), also referred to as INZ-326-141, was 95 to 97 percent pure. The test material was a product of the Sponsor, E.I. duPont de Nemours and Co., Inc.

METHODS:

Bacterial Strains: The plate incorporation assay used Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100.

Preparation of S9: The S9 faction was obtained from the livers of 8 to 9 week-old male Charles River CD® rats that had been dosed with 500 mg/kg Arochlor® 1254 five days before their sacrifice. The S9 fraction was the 9,000 x g supernatant of liver homogenate (1 g wet weight liver in 1.5 ml phosphate buffered saline). Each ml of the S9 mix contained 0.3 ml of the S9 fraction, 0.7 ml of a cofactor mix which contained 8 micromoles $MgCl_2$, 33 micromoles KCl, 5 micromoles glucose-6-phosphate, 4 micromoles NADP, and 100 micromoles sodium phosphate.

Preparation of the Test Material: The test material was dissolved in DMSO and aliquots of this solution were applied to test plates to achieve dose levels of 0.5, 0.75, 1.0, 2.5, 5.0, 10, 50, and 100 µg/ plate. No concentration or stability analyses were performed on the test material and/or solvent solutions.

Controls: DMSO was used as the solvent (negative) control. The following compounds, solubilized in DMSO, were used as positive controls.

Compound	Dose Levels (µg/plate)	Metabolic Activation	Strain Tested
N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	4	-	TA1535, TA100
2-aminoacridine	2	+	TA1535, TA1537, TA98, TA100
9-aminoacridine	50	-	TA1537
2-nitrofluorene	25	-	TA98

Plate Incorporation Assay: The assay was performed using all four bacterial strains without metabolic activation at test material levels of 0.5, 0.75, 1.0, 2.5, and 5.0 µg/plate. All four bacterial strains were assayed with metabolic activation at test material levels of 1, 5, 10, 50 and 100 µg/plate.

Treatments without activation were made by adding 0.1 ml of the solvent or test material solution to an overnight culture containing 10^8 bacteria and 2 ml of top agar. These were mixed and overlayed on Davis minimal agar. For the assay with metabolic activation, 0.5 ml of the S9 mix was added to the mixture of bacteria, test material (or solvent) and top agar, and poured over the minimal agar plates. After incubation at 37° C for 48 hours, all revertant colonies were counted.

Duplicate plates were made for each trial. Two trials were performed for each strain and dose level. The authors did not state whether Trials 1 and 2 were run concurrently. In trials where the results were equivocal or where a positive mutagenic response was obtained, a third trial was performed.

Cytotoxicity: A cytotoxicity test was conducted using dose levels of Linuron up to 10,000 µg/plate. Methods used in this experiment were similar to that of the mutagenesis testing phase of the study except that 10^3 bacteria per plate were used and excess histidine was present. Linuron was shown to be cytotoxic at concentrations of $\geq$ 5 µg/plate without activation and $\geq$ 50 µg/plate with activation. For this reason, slightly toxic to nontoxic concentrations were chosen for the mutagenicity study.

Evaluation Criteria: Two types of analyses were performed to evaluate the data collected. A t-test of significance was used to determine the statistical difference in mutation frequency between test material treated

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groups and the solvent (negative) control. An F-test of significance was used to detect dose response effects.

The criteria used for mutagenicity required that one or more dose levels of the test material should produce a significantly increased number ($p<0.01$) of revertant colonies compared to the negative controls. A positive correlation ($p < 0.01$) between the number of revertants and the increasing dose levels of the test material was also required.

RESULTS:

Mutagenicity: The author reported that with the exception of strains TA1535 and TA100 in the absence of an activation system in Trial 2, no significant increases in revertant colonies or positive linear dose responses were observed. A third trial with strains TA1535 and TA100 without metabolic activation was performed. Results showed that for strains TA1535 and TA100, no increased frequencies of revertant colonies or linear dose responses were present in two of three trials.

Positive mutagenic responses were reported for all assays and tester strains using the positive controls. Revertant colony counts for the positive control, MNNG, without activation averaged 3330 for strain TA1535, and 2107 for strain TA100. Revertant colony counts averaged 1830 for the positive control 2-nitrofluorene without activation in strain TA98. The positive control, 2-aminoanthracene, with S9 induced an average of 1766 revertant colonies in strain TA98 and 718 revertant colonies in strain TA100.

DISCUSSION:

The authors, using a t-test for detecting possible statistically significant differences, concluded that Linuron was nonmutagenic in Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 under these study conditions.

It is our assessment that the author's interpretation of the data was correct. Although the final conclusion in this study was not affected, use of a t-test to evaluate data is too rigorous for this assay. Linuron was not shown to be mutagenic in the tested strains under the study conditions: By using the modified two-fold rule for evaluation, none of the test material treated plates had twice the number of revertant colonies as compared to the negative control. Therefore, no positive mutagenic responses were seen.

The solvent control, DMSO, was used as the negative control for comparison with the test material and the positive controls. Although not used, a nonsolvent negative control group might have been useful to determine whether the spontaneous revertants were within published historical ranges. However, since the revertant colony counts for the solvent control were within the expected range, it is considered that a nonsolvent control group was unnecessary for the study.

Revertant colony counts of over 300, as seen in the positive controls' MNNG without activation, 2-nitrofluorene without activation, and 2-aminoanthracene with activation are inaccurate due to crowding. Although reports on Ames assays typically contain high colony counts for positive control substances, values for these controls should have been reported as > 300, or a dilution should have been made if a more accurate count was required.

CONCLUSIONS:

Results indicate that Linuron (INZ-326-141) was nonmutagenic in Salmonella typhimurium strains TA1537, TA1535, TA98 and TA100 under conditions of this assay. Positive control data showed that the level of sensitivity of the assay was adequate to detect a positive mutagenic response in these strains.

CLASSIFICATION: Acceptable.

EPA: 68-01-6561
TASK: 62
September 6, 1984

DATA EVALUATION RECORD

LINURON

Mutagenicity

CITATION: Chromey, N.C. 1983. Unscheduled DNA synthesis/rat hepatocytes in vitro. An unpublished report No. 190-83 prepared by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, for E.I. DuPont de Nemours and Co., Wilmington, DE. Dated June 3, 1983.

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Date: 10 Oct 1984

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DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity - unscheduled DNA synthesis in rat hepatocytes.

CITATION: Chromey, N.C. 1983. Unscheduled DNA synthesis/rat hepatocytes in vitro. An unpublished report No. 190-83 prepared by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, for E.I. DuPont de Nemours and Co., Wilmington, DE. Dated June 3, 1983.

ACCESSION NUMBER: 251571.

MRID NUMBER: 132583.

LABORATORY: Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE.

QUALITY ASSURANCE STATEMENT: Not present for this report.

TEST MATERIAL: The test material was Linuron (N'-(3,4-dichlorophenyl)-N-methyoxy-N-methylurea). Purity was given as 94.5%. The material was provided by DuPont de Nemours and Co., Lot No. T80311-81.

METHODS:

Preparation of Test Material: The test material, Linuron, was diluted in dimethylsulfoxide (DMSO) to form a stock solution which was diluted in turn in DMSO for use in the assay. The volume of these solutions placed into each well was 20 µl. Final concentrations of the test material in trial 1 were 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, 1.0, 10, and 50 mM. In trial 2, the final concentrations were 10^{-2}, 10^{-1}, 1, 10, and 50 mM.

Controls: A solvent control (20 µl of DMSO) and a positive control (1 mM 7, 12-dimethylbenz(a)anthracene [DMBA]) were included in each assay.

Cell Culture: Primary hepatocyte cultures were prepared from livers of 8-week old, 200-300 g male Charles River/Sprague-Dawley rats. The hepatocytes were obtained from the collagenase-perfused livers of rats anesthetized by ip injection with 65 mg/kg sodium pentobarbital. After washing and the determination of cell viability, 5×10^5 cells were seeded onto plastic coverslips in 6-well tissue culture dishes; each well contained 2.0 ml William's Medium E supplemented with 10% fetal bovine serum. The cultures were incubated at 37° C for 2 hours, washed, and used in the assay.

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Unscheduled DNA Synthesis Assay: After the cells had been cultured and washed, the wash solution was removed from the attached cells, 2.0 ml of medium containing 5 µCi/ml tritiated thymidine was added to each well, and 20 µl of the appropriate concentration of test material or control solution were added. Duplicate cultures were used at each concentration. The cultures were then incubated at 37° C for 18 hours. The coverslips were removed, washed, and placed in clean dishes; the nuclei were then swollen by adding 2 ml of 1% sodium citrate. The cells were fixed with ethanol-glacial acetic acid (3:1), and the coverslips were dried, mounted to slides, coated with "NTB" emulsion, and stored in the dark for 8 days. After the slides were developed, the number of nuclear grains and the grains in an equivalently sized cytoplasmic area were counted by measuring the intensity of light refracted by the grains with a microscope photometer. Counts were made on 25 randomly selected nuclei per slide and on 25 of the "highest labeled" cytoplasmic areas adjacent to the nuclei. Net nuclear counts were obtained by subtracting these background values. Net counts were averaged and differences between doses or trials assessed by a two-way analysis of variance. Significant effects were verified in pairwise comparisons at the 0.05 level.

Evaluation Criteria: An assay was considered acceptable if at least 3 dose levels were not toxic to the cells (a dose was considered toxic if there were fewer than 25 analyzable cells per slide) and the counts for the positive control were significantly greater than the solvent control. The criteria for a positive response apparently required that the assay have an acceptable level of cytotoxicity and the counts for the cells treated with the test chemical be statistically significantly higher than the negative control.

RESULTS:

The results, reported as average net nuclear photometer reading, indicated that the test compound was cytotoxic in one of 2 cultures at 1.0 mM in trial 1; no other toxicity was reported. Results from the duplicates of the control cultures were:

Compound	Concentration	Photometer reading ± S.D.[1] Trial 1	Trial 2
DMSO	1%	-0.1 ± 7.7 -0.2 ± 3.9	1.0 ± 8.5 -1.9 ± 4.8
DMBA	1.0 mM	19.4 ± 9.4 27.0 ± 19.9	25.4 ± 15.8 23.6 ± 17.9

[1]The photometer readings should be expressed in net increase in grain counts after background is subtracted.

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None of the results for the the test compound were found to be statistically different from the controls.

DISCUSSION:

The authors concluded that the test material did not induce unscheduled DNA synthesis, but that the positive control, DMBA, produced a positive effect that was statistically significant.

Our assessment is that the authors have correctly interpreted their results. However, the standard deviation values are large, indicating that this assay can produce highly variable responses. This is not unusual for the state-of-the-art of this procedure. Furthermore, the mean and standard deviation values reported could not be verified since individual values for the 25 random samples were not given in this report. Based on the reported mean values and standard deviation, the positive response obtained with DMBA is considered to be significant. There was clearly no dose-related effect or positive response caused by Linuron.

CONCLUSIONS:

Under the conditions of this study, it appears that Linuron did not induce unscheduled DNA synthesis in primary cultures of rat hepatocytes, but that the positive control was active in inducing unscheduled DNA synthesis.

CLASSIFICATION: Acceptable.

EPA: 68-01-6561
TASK: 62
September 5, 1984

DATA EVALUATION RECORD

LINURON

(CHO-HGPRT Assay)

CITATION: McCooey, K.T., Chromey, N.C., Hemingway, R.E. Testing of linuron in the CHO/HGPRT Assay for gene mutation. Unpublished report No. 540-83 by Haskell Laboratory, E.I. duPont de Nemours and Co. Newark DE. Dated December 16, 1983.

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DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity (HGPRT mutation in CHO cells).

CITATION: McCooey, K.T., Chromey, N.C., Hemingway, R.E. Testing of linuron in the CHO/HGPRT Assay for gene mutation. Unpublished report No. 540-83 by Haskell Laboratory, E.I. duPont de Nemours and Co. Newark DE. Dated December 16, 1983.

ACCESSION NUMBER: 252172.

MRID NUMBER: 00137152.

LABORATORY: Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE.

QUALITY ASSURANCE STATEMENT: Not present.

TEST MATERIAL: The test material was technical linuron of 94.5 percent purity, Lot 1N2-326-141. linuron is 3-(3,4-dichlorophenyl)-methoxy-1-methylurea.

METHODS:

Cell Culture: The BH4 clone of the CHO-K1 cell line, shown to be free of mycoplasma, was cultured as monolayers in Ham's F12 medium without hypoxanthine plus 5 percent dialyzed heat-inactivated fetal calf serum. For cytotoxicity and mutagenicity studies, penicillin(50 µ/ml) and streptomycin (50 µg/ml) were added.

Preparation of Test Material: Dimethylsulfoxide (DMSO) was used as the solvent. Twenty microliters of DMSO containing linuron was added to 3 ml of medium to give final concentrations of 0.05, 0.25, 0.35, 0.40, 0.45, and 0.50 mM in a nonactivated assay and 0.25, 0.50, 0.75, 0.90, and 1.0 mM in an S9 activated assay.

The positive control was ethylmethane sulfonate (EMS, 0.35 mM final concentration) in the nonactivated assay and dimethylbenzanthrancene (DMBA, 0.10 mM final concentration) in the S9 activated assay.

S9 Mix: The treatment medium contained in 3 ml: S9 (3 mg protein), 5.6×10^{-3}M $MgCl_2$, 5×10^{-3}M glucose-6-phosphate, 1.5×10^{-3}M NADP, and test test compound in DMSO. Medium was buffered with 2.5×10^{-2} M Hepes (pH 7.2). The S9 homogenates were obtained from liver slices of Aroclor 1254-treated 8-9 week-old Charles River CD male rats. Cytochrome P-450 levels in the microsome fraction were estimated at 2.48 nmol/mg protein.

Cytotoxicity Assays: In the nonactivated assay, 5×10^5 cells were plated/25 cm^2 flask in 5 ml of medium containing various concentrations of the test compound. After incubation for 18-19 hours, the treatment medium was removed, the plates washed with fresh medium, cells removed by trypsinization, and 200 cells were plated in 60 mm dishes (6 dishes/flask). These dishes were incubated for 7 days and the resultant colonies stained and counted. Survival was calculated as percent of cells plated and as percent of solvent control survival.

In the S9 activated assay, initial toxicity tests were performed with test material and 1.0 mg S9 protein/ml of medium. Two concentrations of test material (0.25 mM and 0.5 mM linuron) giving approximately 30 and 70 percent survival relative to solvent control were retested at 0, 0.2, 0.5, and 1.0 mg of S9 protein/ml of medium. The assays were performed as in the nonactivated assay except that cells were incubated for 5 hours with S9 activation and then for 21-25 hours in fresh medium, before being subcultured. Duplicate flasks were used in the S9 activated assays.

Mutagenesis Assay: Assays were conducted just as in the cytotoxicity tests except that 10^6 cells/100 mm dish were subcultured in medium and maintained in exponential growth for 7 days by subculturing 2 times. After the 7-day incubation to allow for phenotypic expression, 2×10^5 cells were plated (five-100 mm dishes/trial) in medium containing 1×10^{-5} M 6-thioguanine. Colonies were stained and counted after 7 days of incubation. An aliquot of the cells was also plated as described above (cytotoxicity assay) on thioguanine-free medium to measure concurrent survival. Mutant frequency was calculated as number of mutant colonies/10^6 surviving cells.

Data Evaluation: Mutation frequency data were transformed by the formula $Y = (\text{mutant frequency} + 1.00)^{0.15}$. Analysis of variance was performed using both dose and experiment. The student t-test was used to compare mutant frequency. Dose-response relationships were also subjected to analysis of variance and linear, quadratic and higher order effects tested by an F-test.

RESULTS:

Two trials were performed in the nonactivated assay, toxicity was observed at 0.45 and 0.50 mM linuron. There was no increase in mutant frequency at at any level tested. The positive control had a mutant frequency in 4 replicates of 134, 106, 46, and 86 per 10^6 surviving cells, the negative control frequencies were 4.5, 31.7, 5.1, and 1.2 per 10^6 surviving cells, and 0.5 mM linuron gave frequencies of 5.5., 4.3, 14.2, and 33.3 per 10^6 surviving cells.

The S9 activated assay was performed at 0.5 and 1.0 mg S9 protein/ml. Toxicity was observed at linuron levels of 0.75 mM and above with 0.5 mg S9 and at linuron levels of 0.5 mM and above with 1.0 mg S9/ml medium. None of the treatments with the test compound increased in mutant frequencies compared to solvent controls. The positive control DMBA (0.1 mM)

induced mutant frequencies of approximately 400 per 10^6 cells with 0.5 mg S9 and approximately 760 per 10^6 cells at the same dose using 1.0 mg S9/ml medium.

DISCUSSION:

The authors concluded that there was no statistically significant increase over controls in mutant frequency of cells treated at levels of up to 0.50 mM linuron in the nonactivated assay and up to 1.0 mM linuron in the S9 activated assay using concentrations of 0.5 or 1.0 mg S9 protein/ml of medium. There was no significant positive linear dose response in either the nonactivated or S9 activated assays.

Our assessment is that the conclusions are supported by the data. A slightly higher dose could have been used in the nonactivated assay since cell survival at 0.5 mM linuron was 34.8, 67.3, 52.6, and 10.6 percent. At the higher doses of test material, particularly in S9-activated assay, it was not feasible to subculture cells because cytotoxicity reduced their numbers significantly; therefore the media was changed once or twice during the phenotypic expression period. Use of the procedure, however, did not compromise the data. The data with the positive controls showed that the assay was sensitive in detecting mutants at 0.35 mM EMS and 0.10 mM DMBA in the nonactivated and S9 activated assays, respectively.

CONCLUSIONS:

Under conditions of the assay linuron was not mutagenic in the HGPRT Locus of CHO cells in the presence or absence of an S9 activation system.

CLASSIFICATION: Acceptable.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA: 68-01-6561
TASK: 62
September 6, 1984

DATA EVALUATION RECORD

LINURON

Mutagenicity

CITATION: Farrow, M.G., Cortina, T., and Padilla-Nash, H. In vivo bone marrow study in rats with H#14,703. An unpublished study prepared for E.I. duPont de Nemours and Co. by Hazelton Laboratories America Inc. Dated September 1, 1983.

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Date: 10 Oct 1984

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity: In vivo bone marrow chromosome study in rats.

CITATION: Farrow, M.G., Cortina, T., and Padilla-Nash, H. In vivo bone marrow study in rats with H#14,703. An unpublished study prepared for E.I. duPont de Nemours and Co. by Hazelton Laboratories America Inc. Dated September 1, 1983.

ACCESSION NUMBER: 252172.

MRID NUMBER: 00137153.

LABORATORY: Hazelton Laboratories America Inc., Vienna, VA.

QUALITY ASSURANCE STATEMENT: Present, signed and dated 9/2/83.

TEST MATERIAL: The test material, an off-white solid designated H#14,703 had a purity of 94.5 percent active ingredient. The chemical was identified as linuron, N'-(3,4-dichlorophenyl)-N-methoxy N-methylurea.

METHODS:

Animal Species: Sixty-two day old Sprague-Dawley albino rats (95/sex) from Charles River Breeding laboratories were used in the test. Twenty animals/sex were used in the vehicle control group and at each dosing level and 5 animals/group were used in the positive control group. Animals were acclimated to laboratory conditions for 12 days prior to initiation. They were caged individually and offered food and water ad libitum. Laboratory rooms were temperature (70-74° F) and humidity (44-62%) controlled, and maintained on a 12-hour dark/light cycle.

Controls and Mitotic Inhibitors: Vehicle control groups received corn oil. The positive control, cyclophosphamide, was dissolved in corn oil at 2 mg/ml. Colchicine was dissolved in Hanks Balanced Salt Solution (HBBS) at 0.4 mg/ml.

Preparation of Test Material: Linuron was dissolved in corn oil to give stock solutions containing 5, 15, and 50 mg/ml from which animals were dosed by gavage at 20 ml/kg so as to receive 100, 300 or 1000 mg/kg, respectively.

Animal Phase: Groups of 20 animals/sex received a single oral dose of test material at levels of 100, 300, or 1000 mg/kg, the vehicle control group (20 males and 20 females) received a single oral dose of corn oil

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(20 ml/kg) and the positive control group (5 males and 5 females) received a single oral dose of 40 mg/kg cyclophosphamide. The animals received a single intraperitoneal injection of colchicine (2.0 mg/kg body weight) two hours before scheduled sacrifice. Five animals/sex/group were sacrificed at 6, 12, 24, and 48 hours (vehicle control and linuron dosed groups); 5 animals/sex in the positive control group were sacrificed only at 48 hours. After sacrifice by CO_2 asphyxiation, bone marrow cells were collected from each femur by aspiration into 5 ml HBBS prewarmed to 38° C.

Cytogenetic Phase: Bone marrow cells were obtained from the suspension described above by centrifugation at 1100 rpm for 5 minutes, suspended in 5 ml 0.075 M KCl and incubated at 37° C for 25 minutes. Five drops of fixative (methanol:acetic acid, 3:1) were added, the tubes recentrifuged, 5 ml of cold fixative added, the cells recentrifuged and suspended in 0.5-2.0 ml of fixative. Several drops of the suspension were deposited on clean slides and air dried. The slides were stained for 10 minutes with a 4 percent solution of Giemsa, rinsed 2x with distilled water, air dried and mounted with coverslips.

The slides were scanned with a low power objective to find metaphase cells and then the chromosomes were analyzed with a high power oil immersion lens. At least 50 cells were examined from each rat that had analyzable cells. The following data were recorded: number of cells analyzed, number of aberrant cells, chromatid gaps, chromosome gaps, chromatid break, chromosomal breaks, and exchanges, number of cells with greater than 10 aberrations, mean modal numbers of chromosomes, mitotic index, and vernier location of the cells analyzed.

Evaluation of Data: For each group, the mean mitotic index, mean modal number, percent of aberrant cells and total aberrations per animal were statistically compared using the Kruskal-Wallis nonparametric analysis of variance and nonparametric pairwise group comparisons. Although chromosome and chromatid gaps were recorded they were not included in statistical analysis of aberrations.

RESULTS:

Mortality and Clinical Observations: One animal in the 24-hour group dosed at 1000 ppm was found dead and 8 of 10 in the 48-hour group dosed at 1000 ppm died prior to sacrifice on day 2. Signs of toxicity were noted in the low-dose and mid-dose animals ranging from slight depression to ataxia and prostration. There were also significant decreases in body weights when dosed animals were compared to controls. Toxic signs were not observed in positive control animals.

Cytogenetic Analysis: No analyzable metaphase spreads were found for 32 animals in several study groups, however, these slides were randomly distributed between all groups.

No statistically significant increase in the frequency of aberrations occurred in any group dosed with linuron when compared to controls. Values ranged from 0.3-0.8 percent aberrant cells/group. The positive control group had 19.6 percent aberrant cells and 0.602 aberrations/cell. This was a statistically significant increase over controls in percent aberrations at a p value of 0.0001.

The average number of chromosomes in the linuron dosed groups were not significantly different than controls nor was there any change in the mitotic index in dosed groups compared to controls.

DISCUSSION:

The authors concluded that under the conditions of the study linuron was not clastogenic at any of the dose levels tested.

Our assessment is that the data was correctly interpreted by the authors. The sensitivity of the assay was shown by the statistically significant increase in response of the positive control group. Because of excessive toxicity at the high dose, however, the study did not have 3 analyzable dose levels groups.

CONCLUSIONS:

The acute administration of 100, 300, and 1000 mg/kg body weight of linuron to male and female rats caused no significant increase in frequency of chromosomal aberrations and no effects on the modal number of chromosomes or mitotic indices. The test compound was toxic at all levels and caused deaths in 9/10 animals at 1000 ppm.

CLASSIFICATION: Acceptable.